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To: Ed Hammer, USEPA Region 5, Chicago, IL

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Subject: Columbia Environmental Research Center (CERC) preliminary summary for acute sodium chloride toxicity tests with select freshwater organisms

cc: Rip Shively (CERC), Candice Bauer, Dave Mount, Russ Hockett, Sandy Raimondo (USEPA), David Soucek (INHS), Tom Augspurger (USFWS)

This memo contains a preliminary summary of the acute 96-hour sodium chloride toxicity tests with select freshwater mussels conducted at our laboratory over the past 6 years. Please let us know if you have any questions concerning this preliminary summary or if you need any additional information (Ning Wang: 573/441-2946, nwang@usgs.gov).

Preliminary summary of acute water-only sodium chloride toxicity tests conducted with juvenile freshwater mussels

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Date: February 12, 2013

Introduction:

Thirteen reference toxicant (NaCl) tests were conducted at the CERC with juveniles of 5 freshwater mussel species for documenting the quality of test mussels used for our various studies conducted between 2007 and 2011. In 2012, we also conducted 4 acute NaCl toxicity tests with juveniles of 3 mussel species from different tribes of the family Unionidae and a species of the family Margaritiferidae in a study project with USEPA Region 5 (USGS CERC 12-20-03; Acute water-only toxicity of up to 20 chemicals to select freshwater mussels and freshwater snails, and to a cladoceran *Ceriodaphnia dubia* and an amphipod *Hyalella azteca*). Here we provide a summary of the NaCl toxicity data and test conditions that may be useful for the USEPA to update ambient water quality criteria for chloride.

Test conditions and water quality:

All tests were conducted following standard methods outlined in ASTM (2012a). Test conditions for conducting the toxicity tests are summarized in Table 1. Juvenile mussels (a few days old to about 4 months old) were obtained from various mussel culture facilities in Missouri, North Carolina, Virginia, and Wisconsin. The mussels were acclimated to test water (control water) and temperature for at least 48 hours before the start of toxicity testing. The test organisms were fed an algal mixture (Wang et al. 2007) during the acclimation period.

The ASTM reconstituted hard water was used as test water in the NaCl reference toxicant tests conducted between 2007 and 2011, and the CERC diluted well water (100 hard water) was used in 2012 NaCl toxicity tests. The ASTM hard water was prepared by adding reagent-grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$; EM Science, Gibbstown, NJ, USA) into deionized water following ASTM guidance (ASTM 2012b). The 100 hard water was prepared by diluting well water (about 300 mg/L as $CaCO_3$) with deionized water to a hardness of about 100 mg/L as $CaCO_3$. The historical measurements of major cations and major anions in the two test waters are provided in Table 1. Laboratory-grade NaCl (99.9% purity, Sigma-Aldrich, St. Louis, MO) was used to prepared NaCl concentrations. The solution of the highest exposure concentration (8 or 16 g NaCl/L) was prepared by adding 8 or 16 g NaCl into 1000 ml of test water in a 4-L glass jar. A half of the solution was then used for 50% dilutions to create other solutions of lower exposure concentrations.

At the beginning of the 96-h static-renewal test, five organisms were impartially transferred into each of four replicate 50-ml glass beakers containing about 30 ml of water. Test organisms were not fed during the acute exposure. Test beakers were covered with plastic wrap to reduce evaporation and held in a water bath at 20 or 23°C. About 75% water in each replicate beaker was removed and renewed after 48 hours. Water temperature was monitored daily. Water quality (dissolved oxygen, pH, hardness, and

alkalinity) were determined in the control, medium, and high concentrations at the beginning and the end of each test. Mean water quality characteristics are summarized in Table 2. The criterion for establishing effects of chemicals was no foot movement within 5 minutes. The acceptability criterion for a toxicity test is ≥90% control survival.

NaCl concentrations were not measured in most of studies. Salinity and conductivity were measured at the beginning and the end of each test to confirm the NaCl concentrations. The measured salinity and conductivity values were relatively consistent across all tests in the ASTM reconstituted hard water or in the 100 hard diluted well water (Table 3). In addition, we measured chloride concentrations in two NaCl toxicity tests at the beginning of the test (Table 4). The measured chloride concentrations in two NaCl tests were similar to the nominal concentrations; the percent nominal concentrations ranged from 93 to 114% (Table 4). Therefore, we believe that the exposure concentrations in all tests were close to the nominal concentrations.

Nominal NaCl concentrations were used for calculation of median effect concentrations (EC50s) for NaCl, except for the two tests with measured chloride concentrations where EC50s for NaCl were calculated based on measured chloride concentrations. The EC50s for tests conducted between 2007 and 2011 were calculated with TOXSTAT® software (Western EcoSystems 1996) using a Probit model (USEPA 2002). If the data did not meet the requirements of the Probit model, either a Spearman-Karber or trimmed Spearman-Karber method was used (USEPA 2002). The EC50s for tests conducted in 2012 were determined using Toxicity Relationship Analysis Program (TRAP; Erickson 2012).

Preliminary results:

Control survival was ≥90% for all tests, except for one with 85% control survival (Table 2). The EC50s ranged from 2.1 to 5.2 mg NaCl/L among the 17 tests (Table 2). The EC50s for young juveniles (4-day to about 2 weeks old) were generally lower (mean 2.7 mg NaCl/L) than the EC50s for about 2- to 4-month-old juveniles (mean 3.6 mg NaCl/L).

References cited:

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US Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving water to freshwater and marine organisms. EPA/600/4-90/027F, Cincinnati, Ohio.

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mussels (fatmucket, *Lamsilis siliquoidea*) in natural and reconstituted waters. Environmental Toxicology and Chemistry, 28:2367-2377.
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Table 1. Summary of test conditions for conducting NaCl reference toxicant tests with juvenile mussels in basic accordance with ASTM (2012a).

Test chemicals:	NaCl
Test type:	Static renewal
Test Duration:	96 h
Temperature:	20 or 23°C
Light quality:	Ambient laboratory light
Light intensity:	200 or 500 lux
Photoperiod:	16L:8D
Test chamber size:	50 ml
Test solution volume:	30 ml
Renewal of solution:	After 48 h
Age of test organism:	4 days to about 4 months post transformation
No. organisms per test chamber:	5
No. replicate chambers per concentration:	4 (3 replicates in 2 tests due to limited number of mussels)
Feeding:	None
Chamber cleaning:	None
Aeration:	None
Dilution water:	ASTM reconstituted hard water (160-180 mg/L as $CaCO_3$; major cations and anions from historic measurements: mg/L: Ca about 31, Mg 21, K 4.5, Na 51, Cl 6.2, SO_4 188, and dissolved organic carbon 0.3; Wang et al. 2009); or 100 hard water (100 mg/L as $CaCO_3$; mg/L: Ca about 26, Mg 9, K 1.0, Na 9, Cl 10, SO_4 19, and dissolved organic carbon 0.5)
Dilution factor:	0.5
Test concentrations:	0, 0.5,1, 2, 4, and 8 g NaCl/L; or 0, 1, 2, 4, 8, and 16 g NaCl/L
Chemical residues:	Salinity and conductivity were measured at all exposure concentrations at the beginning and the end of test; chloride was measured in select tests.
Water quality:	Dissolved oxygen, pH, hardness, and alkalinity were determined at the control, medium, and high concentrations at the beginning and the end of test
Endpoint:	Survival (foot movement)
Test acceptability criterion:	≥90% control survival

Table 2. Mean water quality characteristics (n=4-6, standard deviation in parenthesis), control survival, and EC50s in acute 96-hour NaCl toxicity tests with juvenile mussels conducted in the ASTM reconstituted hard water or CERC diluted 100 hard well water

Study Code	Test date	Common Name	Scientific Name	Age	# replicate	# org. per replicate	Test Water	Temp. (C°)	Dissolved oxygen (mg/L)	pH	Hardness (mg/L)	Alkalinity (mg/L)	Control Survival (%)	NaCl EC50 (95% CI; g/L)[a]
07-20-18	7/27/07	Rainbow mussel	Villosa iris	2 months	4	5	ASTM hard	20	8.5 (0.5)	8.6 (0.1)	176 (2.2)	121 (2.2)	100	2.5 (2.3-2.9)
08-20-10	5/25/08	Rainbow mussel	*Villosa iris*	2 months	4	5	ASTM hard	20	8.0 (0.3)	8.4 (0.1)	161 (1.0)	120 (2.5)	100	2.7 (2.6-2.9)
08-20-10(2)	8/25/08	Rainbow mussel	*Villosa iris*	2 months	4	5	ASTM hard	20	8.5 (0.2)	8.4 (0.1)	177 (10)	124 (7.4)	100	3.7 (3.2-4.4)
08-20-10(2)	8/25/08	Oyster mussel	*Epioblasma capsaeformis*	2 months	4	5	ASTM hard	20	8.5 (0.2)	8.4 (0.1)	177 (10)	124 (7.4)	100	4.0 (3.4-4.7)
08-20-14	6/24/08	Rainbow mussel	*Villosa iris*	2 months	4	5	ASTM hard	20	8.0 (0.6)	8.1 (0.1)	165 (4.1)	121 (4.7)	100	3.0 (2.8-3.3)
09-20-08	6/22/09	Rainbow mussel	*Villosa iris*	2 months	4	5	ASTM hard	20	8.0 (0.1)	8.4 (0.1)	143 (5.8)	112 (1.9)	90	3.2 (2.8-3.5)
09-20-18	6/30/09	Fatmucket	*Lampsilis siliquoidea*	2 weeks	4	5	ASTM Hard	20	7.6 (0.5)	8.5 (0.1)	192 (4.0)	148 (0.1)	85	2.5 (2.1-2.9)
9-20-21	10/30/09	Fatmucket	*Lampsilis siliquoidea*	2 months	4	5	ASTM Hard	23	8.5 (0.6)	8.4 (0.1)	186 (4.9)	115 (4.3)	100	4.4 (3.8-5.2)
9-20-21	10/30/09	Fatmucket	*Lampsilis siliquoidea*	4 months	4	5	ASTM Hard	23	8.5 (0.6)	8.4 (0.1)	186 (4.9)	115 (4.3)	100	3.7 (3.2-4.3)
10-20-06	2/22/10	Fatmucket	*Lampsilis siliquoidea*	2 months	4	5	ASTM Hard	20	8.5 (0.1)	8.3 (0.1)	165 (4.0)	116 (4.4	100	4.0 (3.4-4.7)
10-20-09	6/21/10	Notched rainbow	*Villosa constricta*	10 days	3	5	ASTM Hard	22	8.3 (0.1)	8.3 (0.1)	172 (4.6)	119 (2.1)	100	3.9 (3.3-4.7)
10-20-09	6/21/10	Yellow lance	*Elliptio lanceolata*	10 days	3	5	ASTM Hard	22	8.3 (0.1)	8.3 (0.1)	174 (6.3)	119 (1.6)	100	2.1 (1.8-2.5)
11-20-07	6/17/11	Notched rainbow	*Villosa constricta*	2 months	4	5	ASTM hard	20	8.8 (0.1)	8.4 (0.1)	169 (1.2)	124 (3.5)	95	5.2 (4.4 - 6.1)
12-20-03(1)	6/14/12	Western pearlshell	*Margaritifera falcata*	4 days	4	5	CERC 100 hard	20	9.2 (0.9)	8.2 (0.2)	101 (1.2)	93.5 (3.0)	100	2.6 (No CI)[b]
12-20-03(1)	6/15/12	Fatmucket	*Lampsilis siliquoidea*	4 days	4	5	CERC 100 hard	20	9.2 (1.0)	8.2 (0.2)	101 (1.2)	93.5 (3.0)	100	2.5 (No CI)[b]
12-20-03(2)	6/28/12	White heelsplitter	*Lasmigona complanata*	6 days	4	5	CERC 100 hard	23	9.8 (1.2)	8.4 (0.2)	111 (2.3)	100 (1.0)	95	2.6 (No CI)[b]
12-20-03(5)	10/29/12	Washboard	*Megalonaisas nervosa*	8 days	4	5	CERC 100 hard	23	8.2 (0.3)	8.5 (0.3)	102 (1.6)	103 (11.9)	95	2.5 (No CI)[b]

[a] EC50 (95% confidence interval) were calculated based on nominal NaCl concentrations except two tests with western pearlshell and white heelsplitter in which EC50s were calculated based on measured chloride concentrations.

[b] No Cl = No confidence limits for EC50 estimate from piecewise regression

Table 3. Mean salinity and conductivity measured at the beginning and the end of acute 96-hour NaCl toxicity tests with juvenile mussels in ASTM reconstituted hard water or in the CERC 100 hard water. The two values measured at the beginning and/or the end of the tests are in a parenthesis

Nominal NaCl (g/L)	ASTM hard water												
	7-20-18 Rainbow mussel	8-20-10 Rainbow mussel	8-20-10 (2) Rainbow mussel	8-20-10 (2) Oyster mussel	8-20-14 Rainbow mussel	9-20-08 Rainbow mussel	9-20-18 Fatmucket	9-20-21 Fatmucket	9-20-21 Fatmucket	10-20-06 Fatmucket	10-20-09 Notched rainbow	10-20-09 Yellow lance	11-20-07 Notched rainblow
Salinity (g/L)													
0	0.05 (<0.01/0.1)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	0.1 (0.1-0.10)	0.05 (<0.01/0.1)	0.05 (<0.01/0.1)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	0.05 (<0.01/0.1)	0.05 (<0.01/0.1)
0.5	0.7 (0.7/0.7)	0.5 (0.5/0.5)	0.6 (0.5/0.6)	0.6 (0.5/0.6)	NT[a]	0.5 (0.5/0.5)	0.6 (0.6/0.6)	0.6 (0.5/0.6)	0.6 (0.5/0.6)	0.6 (0.6/0.6)	NT	NT	NT
1	1.3 (1.2/1.3)	1.1 (1.1/1.1)	1.1 (1.1/1.1)	1.1 (1.1/1.1)	NM[b]	1.1 (1.1/1.1)	1.7 (1.1/2.2)	1.2 (1.1/1.3)	1.2 (1.1/1.3)	1.2 (1.1/ 1.2)	1.2 (1.2/1.2)	1.2 (1.2/1.2)	1.15 (1.2/1.1)
2	2.5 (2.4/2.5)	2.0 (2.0/2.0)	2.8 (2.0/3.6)	2.8 (2.0/3.6)	2.2 (NM/2.2)	2.1 (2.0/2.2)	2.2 (2.1/2.2)	2.2 (2.0/2.4)	2.2 (2.0/2.4)	2.2 (2.1/2.3)	2.2 (2.2/2.2)	2.2 (2.2/2.2)	2.1 (2.1/2.1)
4	4.8(4.6/ 4.9)	4.1 (4.0/4.1)	4.2 (4.0/4.4)	4.2 (4.0/4.4)	4.4 (4.4/NM)	4.5 (4.4/4.5)	4.1 (4.0/4.2)	4.1 (4.0/4.2)	4.1 (4.0/4.2)	4.2 (4.0/4.4)	4.4 (4.4/4.4)	4.4 (4.4/4.4)	4.25 (4.2/4.3)
8	9.3 (9.0/9.5)	8.1 (8.1/8.1)	8.4 (8.1/8.6)	8.4 (8.1/8.6)	8.4 (NM/8.4)	8.8 (8.8/8.8)	8.4 (8.1/8.7)	8.3 (8.0/8.5)	8.3 (8.0/8.5)	8.2 (7.9/ 8.4)	8.6 (8.6/8.6)	8.6 (8.6/8.6)	8.25 (8.2/ 8.3)
16	NT	15.90	NT	NT	16.7 (16.7/NM)	NT	NT	16.6	16.6	NT	16.7 (16.6/16.7)	16.7 (16.7/16.7)	16.3 (16.2/ 16.4)
Conductivity (µS/cm at 25° C)													
0	547 (540/553)	561.5 (552/571)	512 (495/528)	512 (495/528)	631 (593/668)	445 (444/445)	668 (632/704)	642 (627/656)	642 (627/656)	572 (560/584)	602 (597/606)	682 (597/766)	581 (581/580)
0.5	NM	1445 (1442/1447)	1287 (1287/NM)	1287 (1287/NM)	NT	1470 (1458/1482)	1541 (1493/1588)	1508 (1448/1568)	1508 (1448/1568)	1455 (1317/1593)	NT	NT	NT
1	NM	2340 (2330/2350)	2060 (2060/NM)	2060 (2060/NM)	NM	2450 (2440/2460)	2430 (2360/2500)	2510 (2300/2720)	2510 (2300/2720)	2504 (2487/2520)	2510 (NM/2510)	NM	2420 (NM/2420)
2	4290 (NM/4290)	4045 (4030/4060)	4735 (3590/5880)	4735 (3590/5880)	4340 (NM/4340)	3570 (2810/4330)	4175 (4060/4290)	4305 (3999/4610)	4305 (3999/4610)	4368 (4316/4410)	4360 (NM/4360)	NM	4170 (NM/4170)
4	NM	7455 (7420/7490)	6630 (6630/NM)	6630 (6630/NM)	7880 (7880/NM)	7935 (7810/8060)	7505 (7290/7720)	7475 (7280/7670)	7475 (7280/7670)	7886 (7811/7960)	7920 (7890/7950)	7890 (7890/7890)	7640 (NM/7640)
8	14260 (NM/14260)	13980 (13980/13980)	12925 (12550/13300)	12925 (12550/13300)	14320 (NM/14320)	15145 (15120/15170)	14430 (13970/14890)	14260 (14060/14460)	14260 (14060/14460)	14221 (14082/14360)	NM	NM	14107 (NM/14107)
16	NT	27700 (27700/NM)	NT	NT	27000 (27000/NM)	NT	NT	27100	27100	NT	27050 (27000/27100)	26950 (26900/27000)	26700 (NM/26700)

Nominal NaCl (g/L)	CERC 100 hard water			
	12-20-03(1) Western Pearlshell	12-20-03(1) Fatmucket	12-20-03(2) White Heelsplitter	12-20-03(5) Washboard
Salinity (g/L)				
0	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)	<0.01 (<0.01/<0.01)
0.5	NT	NT	NT	NT
1	1.0 (0.9/ 1.0)	1.0 (0.9/ 1.0)	1.1 (1.0/1.1)	1.1 (1.0/1.1)
2	2.0 (1.9/ 2.0)	2.0 (1.9/ 2.0)	2.3 (2.2/2.3)	2.3 (2.2/2.3)
4	4.1 (4.0/4.2)	4.1 (4.0/4.2)	4.1 (4.0/4.2)	4.1 (4.0/4.2)
8	8.3 (8.1/8.4)	8.3 (8.1/8.4)	8.1 (7.9/ 8.3)	8.1 (7.9/ 8.3)
16	17.4 (17.3/17.4)	17.4 (17.3/17.4)	17.6 (17.6/17.7)	17.6 (17.6/17.7)
Conductivity (µS/cm at 25° C)				
0	265 (263/266)	265 (263/266)	320 (260/379)	283 (227/339)
0.5	NT	NT	NT	NT
1	NM	NM	NM	1997 (1604/2390)
2	NM	NM	NM	3390 (2830/3950)
4	7545 (7460/7630)	7545 (7460/7630)	7655 (7770/7540)	6185 (5140/7230)
8	NM	NM	NM	11935 (9830/14040)
16	NM	NM	NM	22445 (18590/26300)

[a] NT = Not tested.

[b] NM = Not measured.

Table 4. Measured NaCl concentrations and percent nominal concentrations in two acute NaCl toxicity tests in the CERC 100 hard water

Nominal NaCl (g/L)	Measured salinity (g/L)	Nominal Cl (g/L)	Measured Cl (g/L)	% nominal Cl
Test with western pearlshell				
0	<0.01	0	0.0093	NA
1	0.95	0.61	0.62	102
2	1.95	1.21	1.28	106
4	4.10	2.43	2.58	106
8	8.25	4.85	4.89	101
16	17.35	9.70	10.10	104
Test with white heelsplitter				
0	<0.01	0	0.0096	NA
1	1.05	0.61	0.691	114
2	2.25	1.21	1.25	103
4	4.10	2.43	2.26	93
8	8.10	4.85	4.63	95
16	17.65	9.70	9.68	100